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OTOLITH ELEMENTAL FINGERPRINTS OF ATLANTIC BLUEFIN TUNA FROM EASTERN AND WESTERN NURSERIES

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SUMMARY

*Otolith chemistry of Atlantic bluefin tuna (*Thunnus thynnus*) was measured to determine the feasibility of the approach for discriminating juveniles (age-0 and age-1) from eastern and western nurseries. Six elements (Li, Mg, Ca, Mn, Sr and Ba) were measured in whole otoliths using solution-based inductively coupled plasma mass spectrometry (ICPMS). Otolith chemistry of age-1* T. thynnus *collected from the two primary nurseries in the Mediterranean Sea and western Atlantic Ocean differed significantly, with cross-validated classification accuracy at 85%. Spatial and temporal variation in otolith chemistry was evaluated for age-0* T. thynnus *collected from three nurseries within the Mediterranean Sea: Alboran Sea (Spain), Ligurian Sea (northern Italy), and Tyrrhenian Sea (southern Italy). Distinct differences in otolith chemistry were detected among Mediterranean nurseries and classification accuracies ranged from 62 to 80%. Interannual trends in otolith chemistry were observed between year classes of age-0 T. thynnus in the Alboran Sea; however, no differences were detected between year classes in the Tyrrhenian Sea. Findings suggest that otolith chemistry of juvenile* T. thynnus *from different nurseries and sub-nurseries are distinct and elemental signatures show some degree of temporal persistence, indicating the technique has considerable potential for use in future assessments of population connectivity and stock structure.*

RÉSUMÉ

*La chimie des otolithes de thon rouge de l'Atlantique (*Thunnus thynnus*) a été mesurée pour déterminer la possibilité de réalisation de l'approche visant à distinguer les juvéniles (âges 0 et 1) des nurseries est et ouest. Six éléments (Li, Mg, Ca, Mn, Sr et Ba) ont été mesurées dans des otolithes entiers au moyen de la spectrométrie de masse plasmique rassemblée par induction et basée sur une solution (ICPMS). La chimie des otolithes du* T. thynnus *d'âge 1 en provenance des deux principales nurseries de la Méditerranée et de l'Atlantique ouest différait de façon significative, avec 85% de précision de la classification une fois contre-vérifiée. La variation spatiale et temporelle de la chimie des otolithes a été évaluée pour le* T. thynnus *d'âge 0 provenant de toris nurseries de la Méditerranée: mer d'Alboran (Espagne), mer Ligure (Italie du nord) et mer Tyrrhénienne (Italie du sud). Des différences de chimie des otolithes ont été détectées entre les nurseries méditerranéennes, et le degré de précision de la classification allait de 62% à 80%. Des tendances inter-annuelles de la chimie des otolithes ont été observées entre les classes annuelles du* T. thynnus *d'âge 0 dans la mer d'Alboran; toutefois, aucune différence n'a été détectée entre les classes annuelles dans la mer Tyrrhénienne. Les résultats laissent entrevoir que la chimie des otolithes du* T. thynnus *juvénile de différentes nurseries et sous-nurseries diffère, et que les signatures élémentaires montrent un certain degré de persistance dans le temps, ce qui indique que la technique a un potentiel considérable quant à son utilisation dans les évaluations futures de la connectivité des populations et de la structure des stocks.*

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RESUMEN

*Se ha medido la química de otolitos del atún rojo del Atlántico (*Thunnus thynnus*) para determinar la viabilidad del enfoque para discriminar a los juveniles (edad-0 y edad-1) de los criaderos del este y el oeste. Se midieron seis elementos (Li, Mg, Ca, Mn, Sr, y Ba) en otolitos completos utilizando espectometría de masa plasmática emparejada de forma inductiva y en solución (ICPMS). La química de los otolitos de* Thunnus thynnus *de edad-1 procedentes de los dos principales criaderos del Mediterráneo y el Atlántico occidental difería de manera significativa, con una precisión en la clasificación, con validación cruzada, del 85%. Se evaluó la variación espacial y temporal en la química de otolitos para* T. thynnus *de edad-0 recopilados de tres criaderos en el Mar Mediterráneo: Mar de Alborán (España), Mar de Liguria (norte de Italia) y Mar Tirreno (sur de Italia). Se detectaron diferencias visibles en la química de otolitos entre los criaderos del Mediterráneo, y las precisiones de la clasificación oscilaron entre el 62% y el 80%. Se observaron tendencias interanuales en la química de otolitos entre las clases anuales de* T. thynnus *de edad 0 en el mar de Alborán; sin embargo, no se detectaron diferencias entre las clases anuales en el Tirreno. Los descubrimientos sugieren que la química de otolitos del* T. thynnus *juvenil de diferentes criaderos y sub-criaderos son huellas distintas y elementales que muestran cierto nivel de persistencia temporal, indicando que la técnica tiene un considerable potencial de uso en futuras evaluaciones de conectividad de población y estructura del stock.*

KEY WORDS

Atlantic bluefin tuna, Otolith chemistry, Stock identification, Mediterranean Sea, Nursery, Trace elements

1. INTRODUCTION

The current two-stock paradigm for Atlantic bluefin tuna (*Thunnus thynnus*) remains unverified, and recent findings suggest that chemical information in otoliths (ear stones) may afford information on stock structure and migratory behaviors of tunas (Rooker et al. 2001b). Otolith chemistry is increasingly used as a technique to differentiate stocks, and interest in its application as a recorder of time and environmental conditions has increased substantially in the past decade (Campana, 1999; Thresher, 1999, Campana and Thorrrold, 2001). Otoliths precipitate as the fish grows and elements from the individual's surroundings are integrated into the aragonite-protein matrix. Since otoliths are metabolically inert, resorption or remobilization of newly deposited elements during ontogeny is negligible. Hence, otolith elemental concentrations may represent reliable natural tags or elemental fingerprints that reflect differences in the chemical composition of the individuals' habitat.

Here, we evaluate the stock-specificity and stability of otolith elemental fingerprints of juvenile *T. thynnus* in western Atlantic Ocean and Mediterranean Sea. Otolith chemistry of juveniles from eastern and western Atlantic stocks (i.e. nurseries) was quantified to determine the discriminatory power of otolith elemental fingerprints for stock discrimination. In order to test for differences between the two primary nurseries, we examined the otolith chemistry of young *T. thynnus* (age-0 and age-1) from the eastern and western Atlantic, and assumed that no transoceanic migration activity occurred. Spatial stability of otolith elemental fingerprints was also examined on a smaller scale by measuring the otolith elemental composition of individuals from putative sub-nurseries within the Mediterranean Sea. In addition, the temporal stability and predictive potential of these natural tags were investigated by contrasting otolith chemistry of two year-classes of age-0 *T. thynnus*.

2. METHODS

Sampling strategies used to procure juvenile (age-0 and age-1) *T. thynnus* varied between regions. In the Mediterranean Sea, age-0 and age-1 individuals were either taken by sport fishermen using hand lines or by commercial long-line fishermen targeting albacore. Collections were made in three

regions of the Mediterranean Sea: Alboran Sea (Spain), Ligurian Sea (northern Italy), and Tyrrhenian Sea (southern Italy). Collections of tuna in the western Atlantic were made in New Jersey and Rhode Island waters using hook and line. Although age-0 and age-1 *T. thynnus* were targeted in both the Mediterranean and western Atlantic, no age-0 *T. thynnus* were collected in western Atlantic during the study period. As a result, our assessment of spatial differences in otolith chemistry between eastern and western stocks was limited to comparisons of age-1 individuals. Collections of age-0 specimens within the Mediterranean Sea were made in both 1998 and 1999 in several regions to evaluate spatial and temporal patterns of otolith chemistry. All collection areas were sampled independently and on more than one occasion. Sizes of age-0 and age-1 bluefin ranged from 25 to 42 and 66 to 70 cm fork length (FL), respectively. In most cases, sagittal otoliths were extracted from freshly caught specimens; however, a small number of samples were frozen prior to otolith extraction. Nevertheless, previous work on *Thunnus* spp. suggests that the effect of short-term freezing on otolith composition is negligible (Rooker et al., 2001a). Selection of single otoliths (i.e. right or left sagittae) for elemental analysis was based on random assignment.

Otoliths were carefully decontaminated according following the protocol described of Rooker et al. (2001a). In preparation for instrumental analysis, each otolith was weighed to the nearest 0.01 mg, placed in a plastic centrifuge tube and dissolved in 10 ml of 1% nitric acid. Internal standards were added to all solutions to compensate for possible instrumental drift. Elemental concentrations were determined using a Perkin-Elmer ELAN 5000 quadrupole inductively coupled plasma mass spectrometer (ICPMS) (Rooker et al. 2001b). Levels of Li, Mg, Mn and Ba were determined using matrix matched external calibration standards; levels of Ca and Sr were quantified after 100-fold dilution using external standards without matrix matching. Samples were analyzed in random order to avoid possible sequence effects. Procedural blanks and two certified reference materials (CRMs).

Multivariate analysis of variance (MANOVA) was used to test for spatial and temporal differences in otolith elemental fingerprints. Nursery ground and year were used as fixed factors in separate MANOVA models. Pillai trace (V) was chosen as the test statistic since it is the most robust to violations of homogeneity of covariance (Wilkinson, 1996). Univariate tests for each element were analyzed using analysis of covariance (ANCOVA). Linear discriminant function analysis (LDFA) was used to classify juveniles from different nurseries and/or year classes. Small differences in otoliths weights and fish lengths occurred among sites and years, and thus elemental concentrations were weight-detrended.

3. RESULTS

3.1 Variability in otolith chemistry between eastern and western stocks

Multivariate analysis of variance indicated that otolith chemistry of age-1 *T. thynnus* collected in Mediterranean and western Atlantic nurseries differed significantly (Pillai's Trace = 3.55, $p < 0.01$). Univariate contrasts indicated that concentrations of only one element (Li) differed significantly (ANCOVA, $p \leq 0.05$) between nursery areas (Fig. 1). Concentration of Li was higher for *T. thynnus* collected in the Mediterranean than in the western Atlantic. Discriminant analysis, based on concentrations of all six elements, indicated that 85% (based on cross-validated or jackknifed classification) of these individuals were correctly assigned to nursery sites (Table 1).

3.2 Variability in otolith chemistry within the Mediterranean

Assessments of within nursery variability in otolith chemistry were conducted for age-0 *T. thynnus*. Results of MANOVA showed that elemental signatures differed significantly among the three putative nurseries within the Mediterranean Sea (Pillai's Trace = 4.32, $p < 0.001$). In addition, univariate contrasts indicated that concentrations of four elements (Li, Mg, Mn, and Sr) differed significantly among the three nurseries (ANCOVA, $p < 0.05$) (Fig. 2). Mg and Mn levels in the otoliths of *T. thynnus* were significantly higher from the Alboran Sea than the Ligurian Sea or Tyrrhenian Sea (Tukey's HSD, $p < 0.05$). Li levels were significantly higher in *T. thynnus* from the

Ligurian Sea while Sr concentrations were significantly lower for individuals from the Ligurian Sea (Tukey's HSD, $p < 0.05$). Results from LDFA indicated that classification success was higher for individuals collected in the Ligurian Sea (80%), and lower for individuals originating from the Alboran Sea (67%) and Tyrrhenian Sea (62%) (Table 1).

3.3 Interannual variability in otolith chemistry

Otolith chemistry of age-0 *T. thynnus* differed significantly between year classes in the Alboran Sea (Pillai's Trace = 10.38, $p < 0.001$). Univariate contrasts indicated that concentrations of three elements (Mg, Mn, Ba) differed significantly (ANCOVA, $p \leq 0.05$) between 1998 and 1999 year classes (Fig. 3). Concentrations of Mg and Mn were higher for age-0 *T. thynnus* in the 1999 group while Ba levels were higher for the 1998 group. Interannual differences in otolith chemistry were not observed between 1998 and 1999 year classes in the Tyrrhenian Sea (Pillai's Trace = 0.282, $p > 0.05$); univariate contrasts for all six elements were statistically similar (ANCOVA, $p > 0.05$). Despite the occurrence of interannual differences in the otolith chemistry of individuals from the Alboran Sea, discriminant analysis showed that cross-validated accuracies of nursery origin (Alboran Sea versus Tyrrhenian Sea) were slightly higher (80%) when year classes were pooled; classification success in 1998 and 1999 between the two nurseries was 78% and 71%, respectively.

4. DISCUSSION

Otolith chemistry of juvenile age-1 *T. thynnus* varied significantly between eastern and western nurseries. However, concentrations of only one element (Li) differed significantly between the two primary nurseries and cross-validated classification success was moderately high (85%). Elemental analysis of age-1 *T. thynnus* collected in other areas of eastern and western nurseries in 1998 has also been recently examined at two different ICPMS facilities (Secor et al., in review) and results were consistent with findings from the present study. Inter-laboratory discrimination of individuals from the two nurseries ranged from 68-81%, and univariate contrasts showed significant differences for Li and Mg at both laboratories. In the present study, a significant site difference was observed for Li but not for Mg; nonetheless, Mg was influential in the classification function, and levels of this element were higher in the Mediterranean as reported in the inter-laboratory study. Differences in otolith chemistry were not entirely unexpected because hydrography and trace element fluxes differ between eastern and western nurseries. The Gulf of Mexico and coastal regions of the western Atlantic are heavily influenced by riverine and terrestrial inputs of metals. Almost half of the riverine discharge of the continental United States flows into the Gulf of Mexico, with the Mississippi River serving as the primary source of anthropogenic and lithophilic elements (Wen et al., 1999). In contrast, the Mediterranean is an evaporite basin and influenced strongly by oceanographic processes (Send et al., 1999). Atlantic waters feed the Mediterranean Sea through the Strait of Gibraltar and supply metal-depauperate water to the region (Minas and Minas, 1993). As a result, elemental concentrations in the Mediterranean Sea are typically lower than in waters of the Gulf of Mexico and coastal areas in the northwest Atlantic Ocean. Still, otolith chemistry of age-1 *T. thynnus* was relatively similar for several elements, suggesting that differences in ambient water chemistry do not completely account for differences in otolith composition.

Differences in otolith chemistry of age-0 *T. thynnus* were also evident within the three putative nurseries of the Mediterranean Sea (Alboran Sea, Ligurian Sea, Tyrrhenian Sea), demonstrating the approach has promise for assessing connectivity or exchange rates among *T. thynnus* from different natal sources within the eastern nursery. Although significant differences were present for Li, Mg, Mn, and Sr, classification success based upon chemical signatures was only moderate (62-80%). Concentrations of Li, Mg, and Mn were higher in the otoliths *T. thynnus* from the northern and western Mediterranean (Alboran Sea, Ligurian Sea) than in the central region (Tyrrhenian Sea), and observed concentrations may be related to oceanographic conditions. Riverine discharge accounts for over 80% of particulate matter in the western Mediterranean (20% due to atmospheric sources), and levels are markedly higher than in the central or eastern Mediterranean (Guerzoni et al., 1999). Consequently, rivers in the northwestern Mediterranean Sea (e.g. Rhône, Ebro) are likely sources of

lithophilic elements. In addition, metal-enriched inputs from the southwest coast of Spain (Tinto and Odiel Rivers) are transported through the Strait of Gibraltar, and mix with waters of the western Mediterranean. The western region is also well fertilized by upwelling at the northern boundaries of the anticyclonic gyre, and concentrations of elements displaying nutrient-type distributions may be higher in these cold, nutrient-rich waters. Thus, elevated concentrations of certain elements in otoliths of juvenile *T. thynnus* collected from this region may be due in part to variations in water chemistry.

Our results suggest that spatial differences in chemical signatures in the otoliths of *T. thynnus* can be used to establish natal origin; nevertheless, classification accuracies were moderate and may need to be improved before full-scale investigations of stock structure are attempted. Enhancing the resolving power of the approach is possible and several options are available. First, preconcentration procedures can be used in the future to eliminate matrix interferences on the ICPMS, allowing analysts to accurately determine transition metal concentrations at nM-pM levels. Second, as demonstrated by Thorrold et al. (2001), stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) may be used in conjunction with trace element chemistry to improve classification success. Stable isotopic ratios have been used extensively as recorders of environmental conditions and integration of $\delta^{13}C$ and $\delta^{18}O$ into future assessments of *T. thynnus* may enhance our resolving power. Third, due to migratory behavior, future assessments of natal origin should be restricted to young-of-the-year (age-0) *T. thynnus*, particularly individuals collected within the first year of life. *Thunnus thynnus* are known to display trans-oceanic migration activity (Magnuson et al., 1996), and therefore it is possible that our reference sample included migratory yearlings from mixed stocks.

Retrospective determination of natal source depends on the premise that chemical signatures are sufficiently stable across time to allow for accurate classification. To date, a limited number of studies have examined the issue of temporal stability of otolith chemistry and results indicate that stock-specific signatures vary among years (Campana et al., 2000). Long-term stability of otolith elemental fingerprints is not evident in any study to date, suggesting that elemental fingerprints may serve only as short-term natural tags (1-3 yrs). Temporal stability of elemental fingerprints of Pacific bluefin tuna (*Thunnus orientalis*) was recently examined over a three-year period in the North Pacific Ocean (Rooker et al., 2001b). Although interannual trends were evident, differences in otolith chemistry between Pacific Ocean and coastal sea nurseries were greater than temporal variability within a nursery. In the present study, otolith composition of *T. thynnus* differed between two year-classes in the Alboran Sea; however, univariate contrasts for all six elements were statistically similar in the Tyrrhenian Sea. Although interannual differences were observed in the Alboran Sea, classification accuracies were not negatively affected. In fact, our ability to discriminate individuals from the Alboran Sea and Tyrrhenian Sea was improved by pooling year classes (cross-validate accuracy of 80%), indicating spatial differences were sufficient to counter temporal variability.

In summary, this study demonstrates that otolith chemistry of juvenile *T. thynnus* from eastern and western nurseries is distinct, and elemental fingerprints show some degree of temporal persistence. Observed natural variability in elemental fingerprints among nurseries suggests the approach has considerable promise for differentiating stocks of *T. thynnus*, and findings from future assessments should shed some light on the issue of population connectivity (mixing) of *T. thynnus.*

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Table 1. Jackknifed (cross-validated) classification accuracies of sagittal elemental concentration data from discriminant analysis. Estimates of percent classified correctly are given for age-1 *Thunnus thynnus* from eastern and western nurseries and age-0 *T. thynnus* from three regional nurseries within the Mediterranean.

Location	No.	% Correct
W. Atlantic	12	83
Mediterranean	8	88
Total	20	85
Alboran Sea	10	75
Ligurian Sea	10	80
Tyrrhenian Sea	21	52
Total	41	65

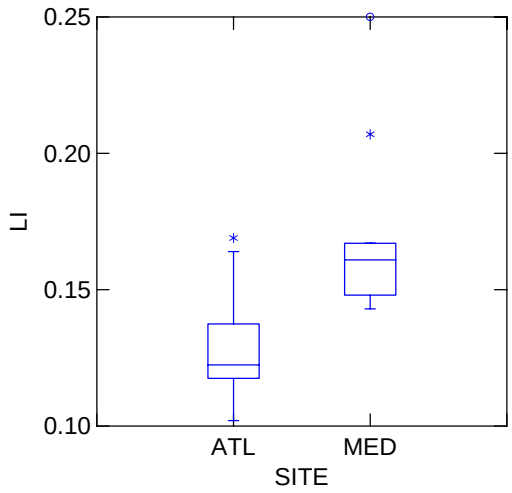

Figure 1. Box plot of Li concentrations of otoliths from age-1 *Thunnus thynnus* collected in 1999 from western Atlantic Mediterranean (Ligurian Sea) nurseries. Interquartile ranges (25th and 75th percentile) are shown by extent of boxes and horizontal lines represent medians (50th percentile). Whiskers range from 10th to 90th percentiles and values outside this range are plotted with asterisks or open circles. Concentrations given in parts per million.

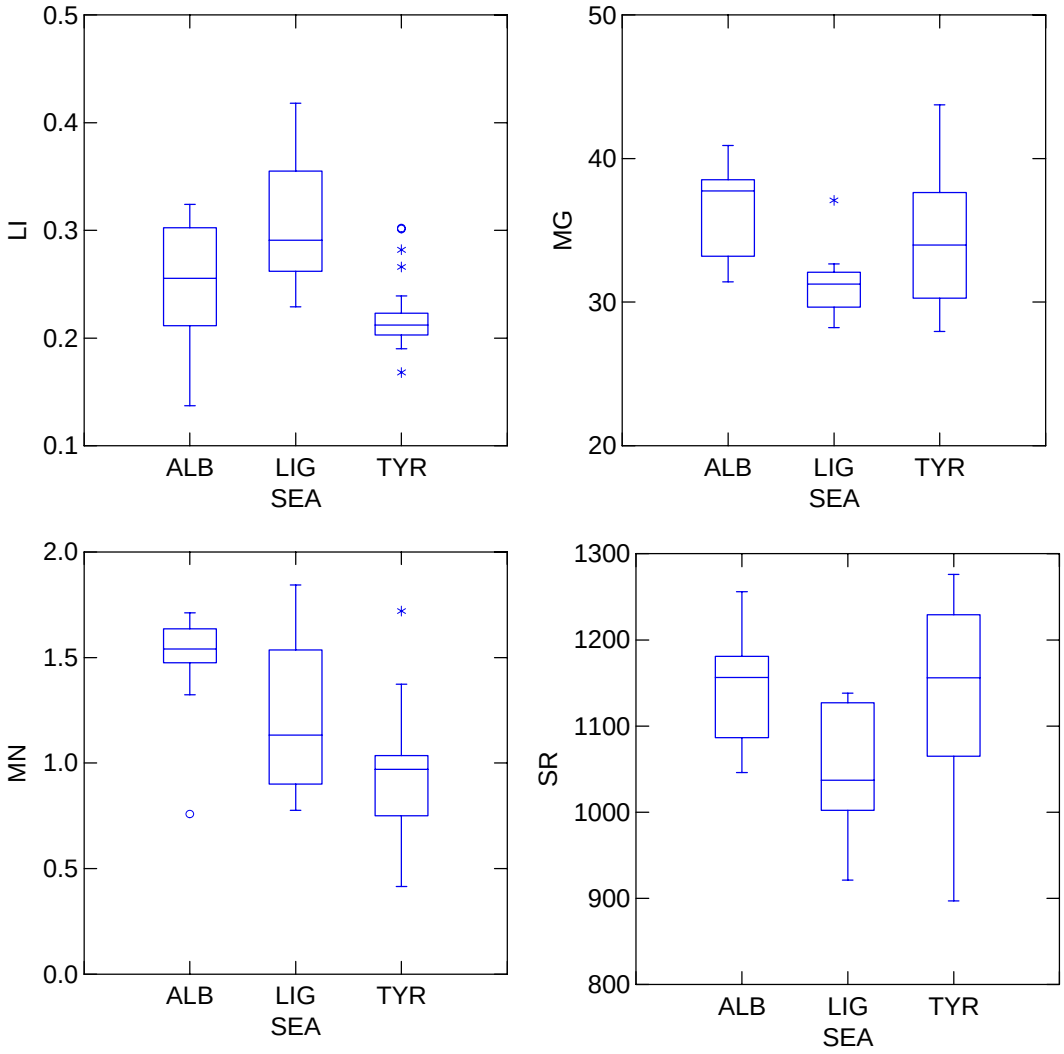

Figure 2. Box plots of elemental concentrations of otoliths from age-0 *Thunnus thynnus* collected in 1999 from three putative nurseries in the Mediterranean Sea: Alboran Sea (ALB), Ligurian Sea (LIG), Tyrrhenian Sea (TYR). Concentrations given in parts per million. Boxes, lines, and whiskers as in Fig. 2.

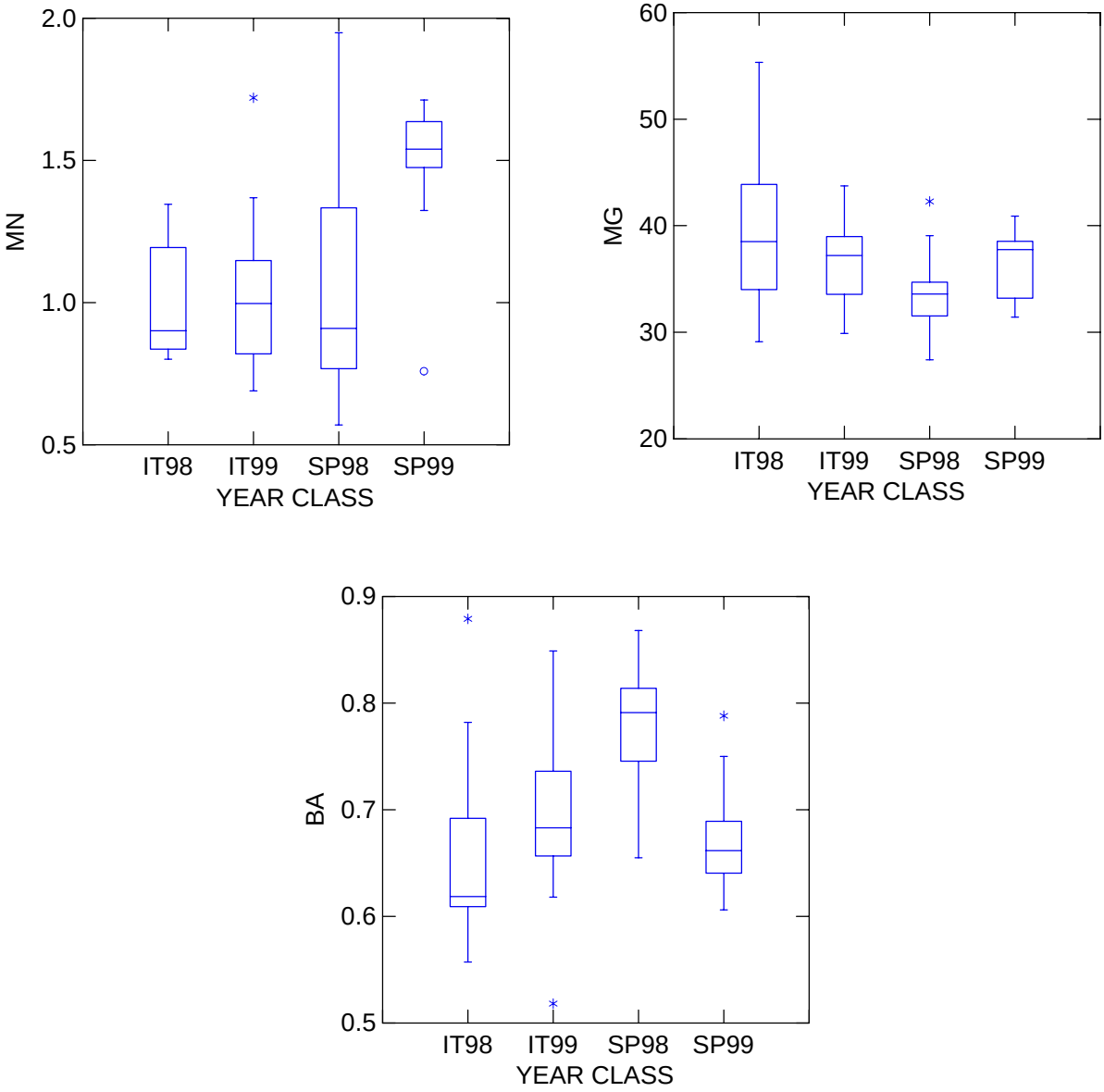

Figure 3. Density plots of elemental concentrations of otoliths from two year classes (1998, 1998) of age-0 *Thunnus thynnus* from two nursery areas in the Mediterranean Sea: Spain (SP)-Alboran Sea and Italy (IT) -Tyrrhenian Sea. Concentrations given in parts per million.